Bovine Abortions and Stillbirths in Denmark 2015 to 2017

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Bovine Abortions and Stillbirths in Denmark 2015 to 2017

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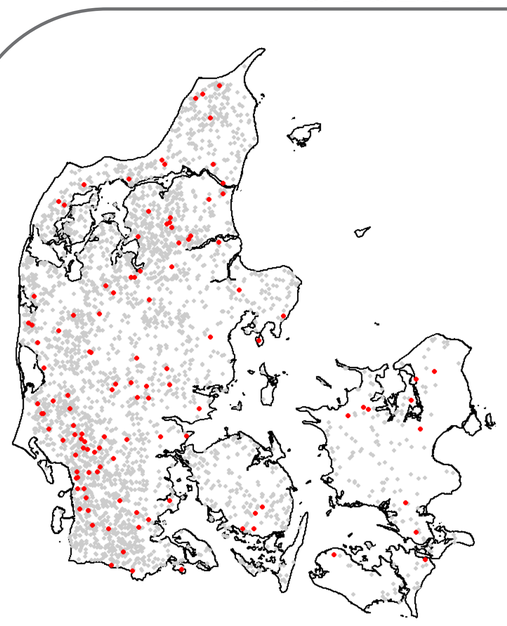
Infections are the most common cause of bovine abortion. Here we report recent diagnostic findings in bovine abortion material from Denmark, a country with a large dairy sector and high animal health standards. This study was conducted in order to gain in-depth knowledge on infectious causes of abortions i.e. to identify and localize infectious agents in placental and foetal tissues. The cultivation-independent methods **Fluorescence *in situ* hybridization (FISH)** and **second generation sequencing** were applied additionally to routine histopathology and bacterial cultivation.

STUDY POPULATION



0.8% of reported abortions during study period

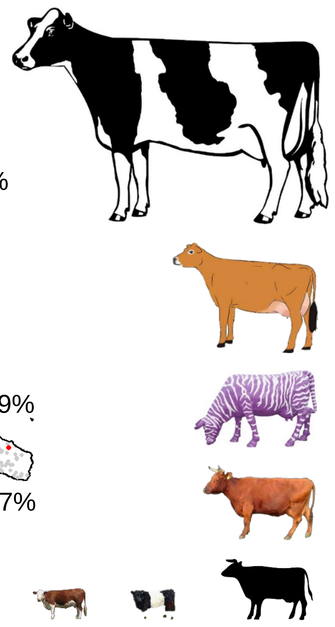


Figure 1. The study population consisted predominantly of dairy cows (5% beef) from mainly conventional farms (9% organic) and originated from across the country matching the geographical distribution of dairy farms in Denmark (map displaying dairy farms as **grey** dots and abortion submitting farms as **red** dots). The majority of the abortions took place during mid to late gestation.

SAMPLE MATERIAL

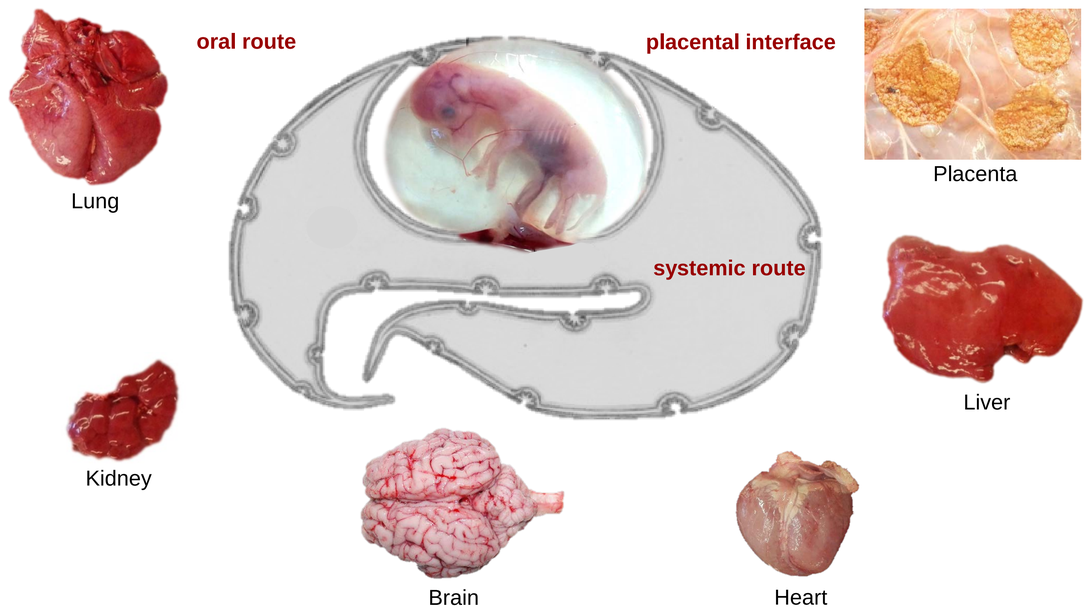


Figure 2. Organs collected at necropsy for histological and molecular analyses. Organs were chosen based on known predelection sites for abortogenic infections (e.g. neosporosis in brain, liver, heart; *Leptospira interrogans* in kidneys) and related to potential ports of entry into the foetus. Abortion material was submitted to DTU Vet for routine abortion diagnostics from January 2015 until June 2017.

FISH

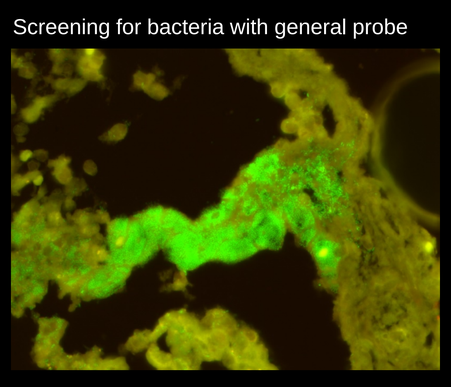


Figure 3. Placenta. Example of a cotelydonary villus with extendend and necrotic trophoblasts containing large numbers of intracytoplasmatic bacteria (green). Bacterial localisation and morphology will be used to choose relevant species-specific probes for further identification of intralesional bacteria.

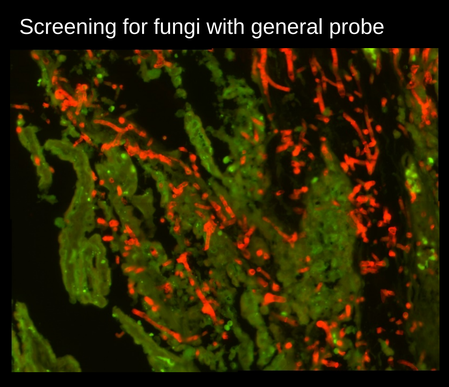


Figure 4. Placenta. Multiple cotelydonary villi invaded by fungal septate and branching hyphae. Fungal structures have been detected within placental lesions in 3% of the investigated placentas using FISH.

SECOND GENERATION SEQUENCING

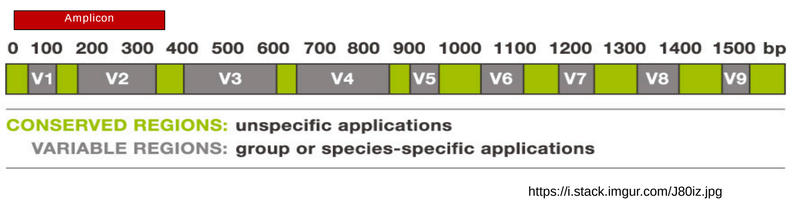


Figure 5. DNA was extracted from placenta, a liver and lung pool as well as kidney. Part of the total bacterial DNA per sample was amplified using universal bacterial primers binding adjacent to the variable regions V1 and V2 of the 16S rRNA gene. The resulting amplicon was sequenced on the Illumina MiSeq platform.

CONCLUSIONS

- **Neosporosis** was the most frequently diagnosed infection.
- **No epizootic abortifacients** were found on study population level, however, due to very few abortions submitted per herd, no conclusions can be drawn on herd level.
- **Fungi** seem to play a minor role as arbortogenic agent in Denmark.

RESULTS

BRUCELLA ABORTUS CULTIVATION

All foetal organ pools were negative for *Brucella abortus.*

HISTOPATHOLOGICAL SCREENING FOR *NEOSPORA CANINUM*

Neosporosis was diagnosed in 30 out of 162 abortions (19%) based on findings in HE stained tissue sections of brain, heart, and liver.

ELISA

In 90% of the cases, a blood sample of the dam was submitted. All samples were negative for maternal BVDV antibodies.

Table 1. Bacterial genuses/species isolated using routine aerobe cultivation.

Species	n	Genus/Species	n	Genus/Species	n	Genus/Species	n
Escherichia coli	37	Aeromonas spp.	7	Listeria monocytogenes	3	Moellerella wisconsensis	1
Acinetobacter spp.	15	Staphylococcus spp.	6	Vibrio spp.	3	Morganella morganii	1
Aerococcus spp.	13	Trueperella pyogenes	5	Vagococcus spp.	3	Pantoea agglomerans	1
Proteus	13	Streptococcus spp.	5	Lactobacillus spp.	2	Pasteurella spp.	1
Lactococcus spp.	9	Bacillus licheniformis	5	Klebsiella spp.	2	Serratia fonticola	1
Enterococcus spp.	8	Hafnia alvei	5	Citrobacter spp.	1	no bacteria isolated	30

n = number of abortions from which genus/species was isolated

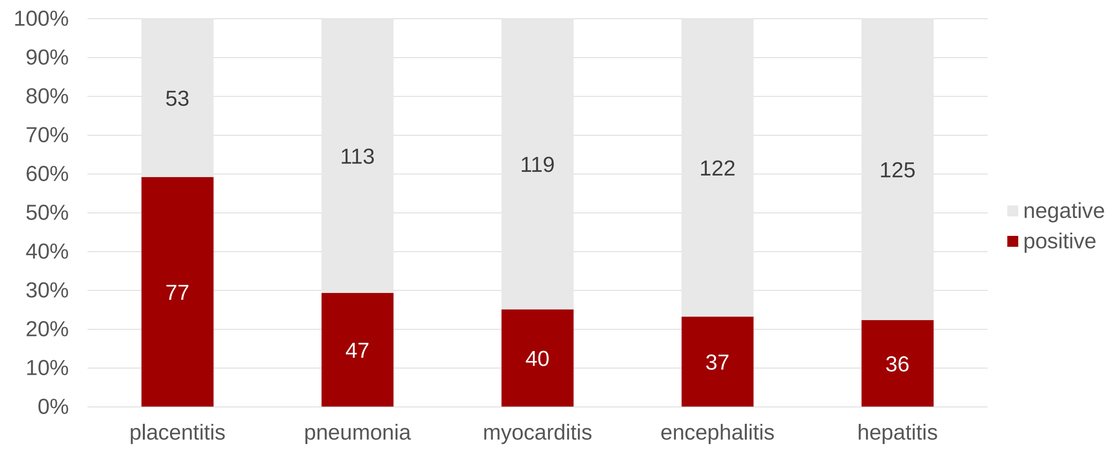


Figure 6. Distribution of organ lesions diagnosed in HE tissue sections shown as number and percentage of abortions.

SECOND GENERATION SEQUENCING

All abortion samples were **negative** for DNA from the following known abortifacients:

- *Chlamydia/Parachlamydia spp.*
- *Brucella abortus*
- *Campylobacter fetus*
- *Pajaroellobacter abortibovis* (epizootic bovine abortion)
- *Listeria ivanovii*

Leptospira interrogans DNA was detected in one liver sample.
Coxiella burnetii DNA was detected in samples from four abortions.

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